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Synthesis of (-)-epigallocatechin-3-gallate derivative containing a triazole ring and combined with cisplatin/paclitaxel inhibits NSCLC cancer cells by decreasing phosphorylation of the EGFR

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Abstract

Non-small-cell lung cancer is one of the principal causes of cancer-related death around the world. Chemotherapy is commonly used to treat wild type of epidermal growth factor receptor non-small-cell lung cancer. (-)-Epigallocatechin-3-gallate is the most abundant and active catechin. However, (-)-epigallocatechin-3-gallate has limited clinical application due to its poor stability and absorption. Herein, we report that a glycosylated azide undergoes a click reaction with the terminal alkyne of (-)-epigallocatechin-3-gallate to yield a triazole-linked glucose-(-)-epigallocatechin-3-gallate derivative and have evaluated its in vitro anticancer activity against human non-small-cell lung cancer cells using the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide assay. The product inhibits human non-small-cell lung cancer cell lines with wild type of epidermal growth factor receptor and in combination with cisplatin/paclitaxel results in more pronounced proliferation inhibition than when used alone. Stability investigations indicates that the conjugated glucose residue can improve the stability of the (-)-epigallocatechin-3-gallate scaffold. Our studies suggest that the combination of the glucose-(-)-epigallocatechin-3-gallate derivative and chemotherapeutic drugs may provide a novel strategy for the treatment of non-small-cell lung cancer.

Keywords

combination, (-)-epigallocatechin-3-gallate derivative, wild type of epidermal growth factor receptor, non-small-cell lung cancer, synthesis

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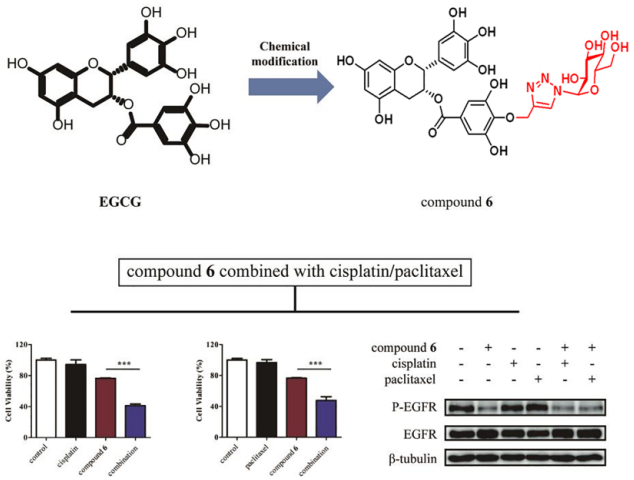
(-)-epigallocatechin-3-gallate derivative combined with cisplatin/paclitaxel inhibits non-small-cell lung cancer cells



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Introduction

Lung cancer is a very common malignancy with thousands of cancer-related deaths reported worldwide, and non-small-cell lung cancer (NSCLC) accounts for 85% of all cases of lung cancer.[1] The epidermal growth factor receptor (EGFR) is a well-established critical target for the treatment of NSCLC.[2] Overexpression of EGFR, as a result of abnormal activation of the anti-apoptotic signaling pathway and abnormal cell proliferation, is known to be important in NSCLC.[3] The epidermal growth factor receptor tyrosine kinase inhibitors (EGFR-TKIs), such as gefitinib and erlotinib, play a key role in treating NSCLC harboring EGFR with activating mutations.[4] Unfortunately, most patients have wild-type EGFR ($_{wt}$EGFR) tumors, and the efficacy of EGFR-TKIs for the treatment of these patients is still contentious.[5] Nevertheless, EGFR-TKIs are approved for second-line treatment of patients with $_{wt}$EGFR tumors.[6] Chemotherapy, as a conventional treatment for many types of cancer, is one of the first-line treatments for NSCLC patients harboring $_{wt}$EGFR.[7,8] However, it has no significant therapeutic effect.[9] Therefore, new therapeutic strategies are urgently required.

Green tea is one of the most popular types of daily beverages worldwide.[10,11] (-)-Epigallocatechin-3-gallate (EGCG), is the most abundant catechin (accounting for approximately 50% of total catechins), is the most active phenolic constituent of green tea catechins and has been extensively reported to treat various cancers including liver, lung, breast, throat, prostate, and bladder cancer.[11–15] A study showed that EGCG can inhibit EGFR tyrosine kinase activity in the A549 human epidermal carcinoma cell line with $_{wt}$EGFR.[16] However, the use of EGCG is often hindered by problems such as easy oxidation, ready degradation in aqueous solutions, and poor intestinal absorbance.[17,18]

In our previous study, we synthesized a series of EGCG glucoside derivatives by a chemical modification strategy and evaluated their antitumor activity.[17,19] Since the 1,2,3-triazole ring is a widespread functional group in many drugs,[20–25] it is of interest to attach 1,2,3-triazoles to EGCG derivatives. Herein, we report the chemical synthesis of a triazole-linked glucose EGCG derivative, its in vitro anticancer activity against human NSCLC cell lines (NCI-H292, NCI-H441, NCI-H1781, A549, and NCI-H1975), and tested this synthetic compound combined with chemotherapeutic drugs (cisplatin/paclitaxel) on proliferation in cancer cells. In addition, we tested the stability of triazole-linked glucose EGCG derivative compared to EGCG.

Results and discussion

Click-chemistry strategy for the synthesis of the EGCG derivative

Glucosylated EGCG (compound **6**) was synthesized according to Scheme 1. The preparation of EGCG terminal alkyne **2** was carried out in 45% yield by the reaction of EGCG (**1**) with sodium hydride and propargyl bromide. D-Glucose was readily converted into glycosylated azide **5** according to a known procedure.[26] Finally, according to a click-chemistry strategy,[27] the glycosylated azide **5** was reacted with alkyne **2** in the presence of copper (II) acetate and sodium ascorbate in *tert*-butyl alcohol/water (1:1) at room temperature for 2 h to afford the EGCG glycoside **6** in 75% yield. The product was characterized by ^{1}H-NMR, ^{13}C-NMR, ESI-MS, and HRESI-MS.

Reagents and conditions: (a) Ac_2O, NaOAc, 100°C, 20 min, 99%; (b) HBr, AcOH, CH_2Cl_2, 0°C, 8 h, 96%; (c) NaN_3, DMF, 12 h, 90%; (d) CH_3ONa, CH_3OH, rt, 12 h, 78%; (e) propargyl bromide, NaH, DMF, 0°C–rt, 12 h, 40%; and (f) copper(II) acetate, sodium ascorbate (1 M, in H_2O), *t*-BuOH-H_2O (1:1), THF, rt, 2 h, 75%.

*Compound **6** decreases cell viability in NSCLC cells*

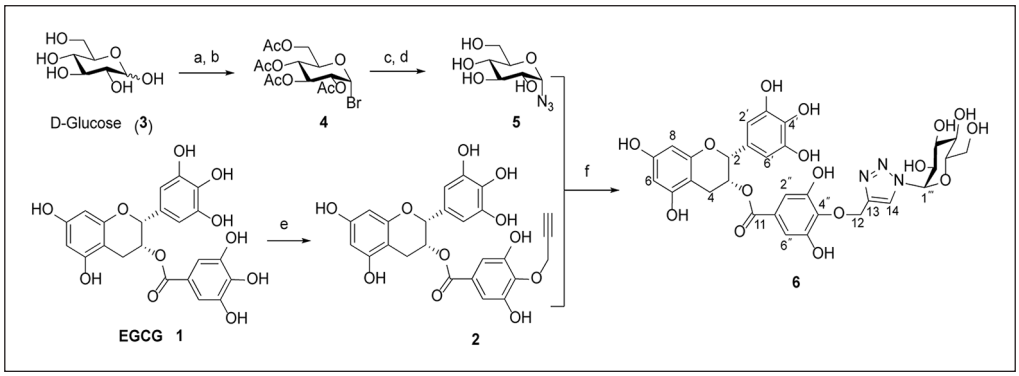
Our previous study suggested that glucosylated EGCG derivatives combined with gefitinib inhibited the proliferation of NSCLC cells (NCI-H1975).[19] To test whether compound **6** inhibits cell proliferation in NSCLC cells, we chose four human NSCLC cell lines harboring $_{wt}$EGFR (NCI-H292, NCI-H441, NCI-H1781, and A549) and one human NSCLC cell line with the L858R/T790M mutation (NCI-H1975) to measure cell viability using the MTT assay (Figure 1). As shown in Figure 1(a)–(d), compound **6** significantly inhibited the cell viability of NCI-H292, NCI-H441, NCI-H1781, and A549 at different concentrations. By contrast, compound **6** did not inhibit the cell viability of NCI-H1975 cells. These results indicate that compound **6** inhibits human NSCLC cell lines with $_{wt}$EGFR compared to the EGFR L858R/T790M mutation.

*Compound **6** combined with cisplatin/paclitaxel decreases cell viability in NCI-H1781 cells*

It has been reported that almost all tumors acquire resistance to chemotherapeutic drugs after periods of treatment.[28,29] Given that compound **6** inhibited NSCLC cells with $_{wt}$EGFR, we hypothesized that compound **6** combined with a chemotherapeutic drug would exhibit greater anticancer activity potential than when used alone. Thus, we treated NCI-H1781 cells with compound **6** (60 μM) alone and in combination with cisplatin/paclitaxel (12 μM) by using a cell proliferation assay (Figure 2). As shown in Figure 2(a) and (b), compound **6** combined with cisplatin resulted in more pronounced proliferation inhibition than **6** or cisplatin alone. The combination of compound **6** and paclitaxel indicated similar proliferation inhibition.

*Compound **6** combined with cisplatin/paclitaxel inhibits phosphorylation of EGFR in NCI-H1781 cells*

It is well known that the EGFR signal pathway plays a key role in the treatment of NSCLC.[19,30] Therefore, we treated NCI-H1781 cells with the combination of compound **6** and cisplatin/paclitaxel and examined the phosphorylation levels of the EGFR by western blotting (WB; Figure 3). As



Scheme 1. Click-chemistry strategy for the synthesis of a triazole-linked glucose EGCG derivative.

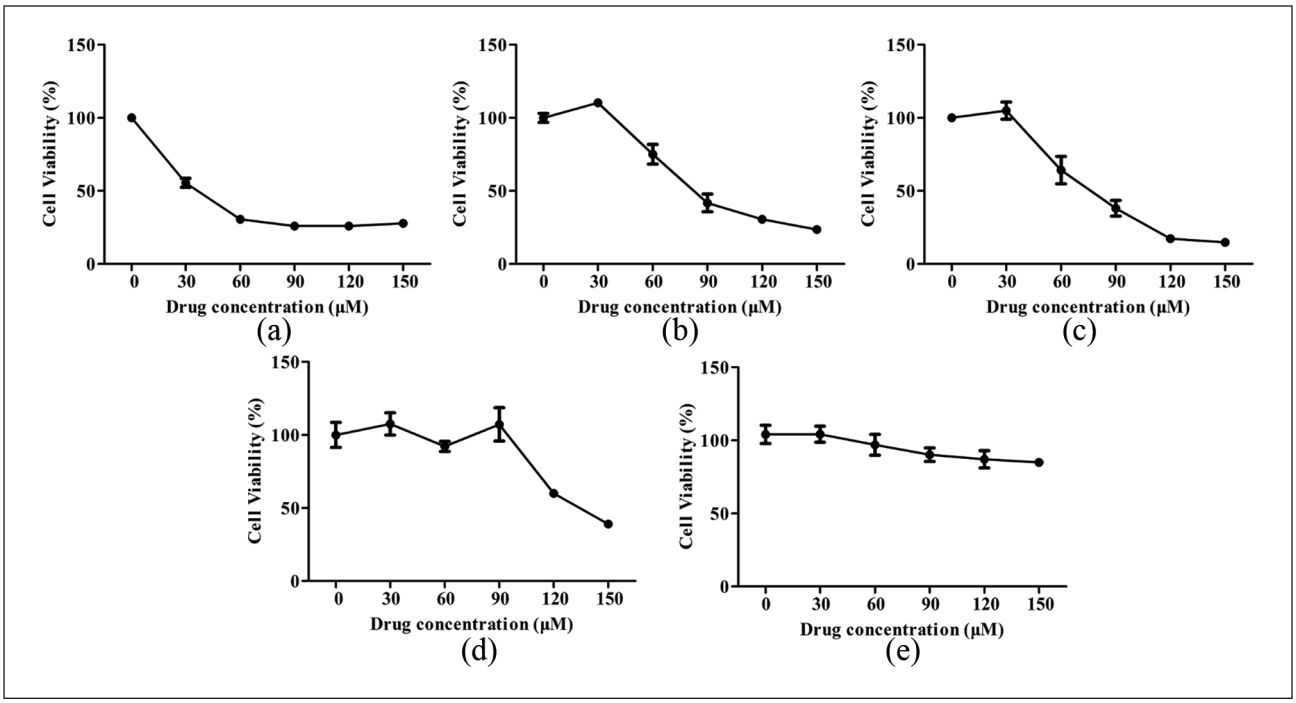


Figure 1. Effect of compound **6** on the proliferation of NSCLC cell lines. (a) NCI-H292, (b) NCI-H441, (c) NCI-H1781, (d) A549, and (e) NCI-H1975. All cells were treated with increasing doses of compound **6** at the indicated concentrations (0, 30, 60, 90, 120, and 150 μM) for 48 h. Cell proliferation was measured by the MTT assay and expressed as the proliferation rate.

shown in Figure 3, treatment of the combination of compound **6** and cisplatin/paclitaxel inhibited the phosphorylation of EGFR more significantly than compound **6** alone, while the total protein levels of EGFR remained unchanged. These results indicate that compound **6** combined with cisplatin/paclitaxel may inhibit proliferation in NCI-H1781 cells through the EGFR signal pathway.

*Compound **6** showed more stability than EGCG*

In our previous study, we found that glucosylated EGCG derivatives had improved chemical stability and absorption compared to EGCG.[17] To investigate whether compound **6** showed more stability than EGCG, we tested the stability of compound **6** and EGCG according to the amount of hydrogen peroxide (H2O2) which is a by-product of biological oxidation processes (Figure 4). Glucosylated EGCG derivative with a triazole ring **6** showed greater stability than EGCG. It appears that incorporation of the glucose moiety slows down the hydrolysis and improves the stability of the EGCG scaffold.

Conclusion

In conclusion, we have used a glycosylated azide in a click reaction with the terminal alkyne of EGCG to yield a triazole-linked glucose-EGCG derivative **6** in high yield. Compound **6** was screened for anticancer activity against a panel of five human NSCLC cells (NCI-H292, NCI-H441, NCI-H1781, A549, and NCI-H1975) and was found to inhibit human NSCLC cell lines with $_{wt}$EGFR compared to the EGFR mutation. In addition, compound **6** combined with cisplatin/paclitaxel resulted in more pronounced proliferation inhibition than when used alone, which may occur through an EGFR signal pathway. Finally, stability

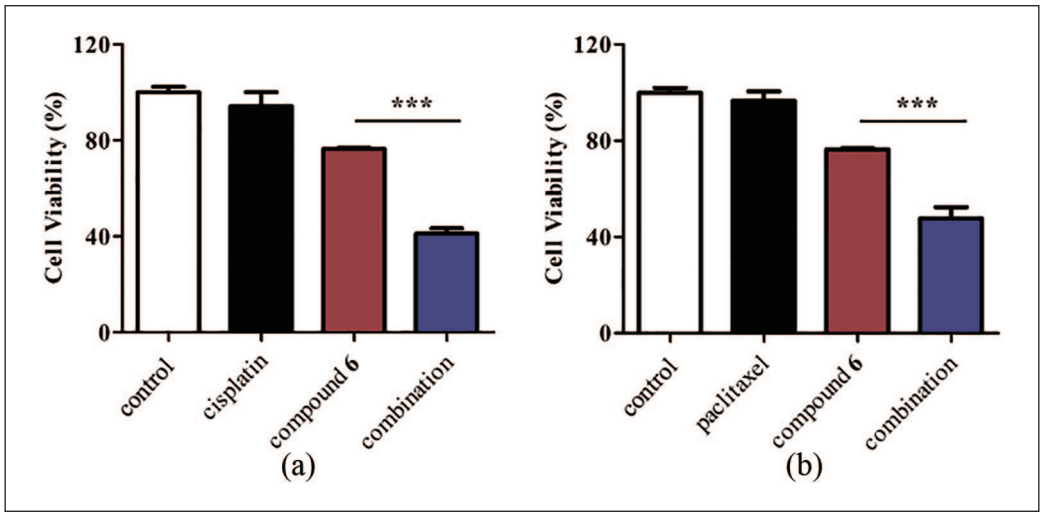


Figure 2. Compound **6** combined with cisplatin/paclitaxel decreases cell viability in NCI-H1781 cells. (a) Compound **6** (60 μM) combined with cisplatin (12 μM) and (b) compound **6** (60 μM) combined with paclitaxel (12 μM). Cell proliferation was measured by the MTT assay and expressed as the proliferation rate. Data represent the average of three independent experiments (mean ± SEM). $^{**}p < 0.01$, $^{***}p < 0.001$.

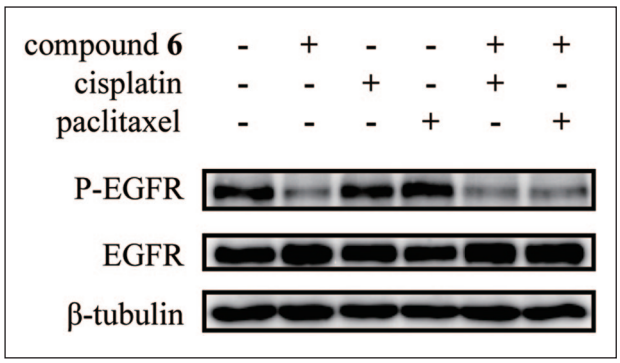


Figure 3. Compound **6** combined with cisplatin/paclitaxel inhibits phosphorylation of EGFR in NCI-H1781 cells. Compound **6** (60 μM), cisplatin/paclitaxel (12 μM) alone, or with compound **6** plus cisplatin/paclitaxel, after 12 h. NCI-H1781 cells were stimulated with 10 ng/mL EGF for 10 min. The expression levels of the proteins were determined by western blotting (WB). β-Tubulin was tested as the loading control.

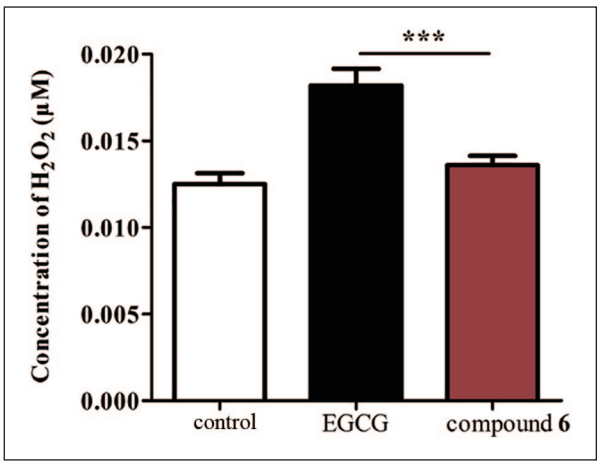


Figure 4. The stability compound **6** according to the amount of hydrogen peroxide (H_2O_2). H_2O_2 concentrations of EGCG and compound **6** were tested using a H_2O_2 Quantitative Assay Kit (water-compatible). $^{***}p < 0.001$. EGCG was taken as the control drug.

investigation indicated that a conjugated glucose residue seems to improve the stability of the EGCG scaffold. Our studies suggest that the combination of compound **6** and chemotherapeutic drugs may provide a novel strategy for the treatment of NSCLC.

Experimental

Materials

EGCG was purchased from Chengdu Proifa Technology Development Co., Ltd (Chengdu, China); D-glucose was purchased from Sinopharm Chemical Reagent Co., Ltd. (Shanghai, China); and cisplatin, paclitaxel, and 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) were obtained from Sigma-Aldrich (St. Louis, MO, USA). All reagents were commercially available and used without further purification unless indicated otherwise. Mass spectrometry (MS) and high-resolution mass spectrometry (HRMS) were performed on an Agilent 6540 Q-Tof ESIMS (Agilent Technologies, Santa Clara, CA, USA). ^{1}H-NMR and ^{13}C-NMR spectra were recorded on a Bruker AVANCE III 500 MHz (Bruker BioSpin GmbH, Rheinstetten, Germany) instrument, using tetramethylsilane (TMS) as an internal standard. Silica gel (200–300 mesh) for column chromatography and silica GF_{254} for thin-layer chromatography (TLC) were produced by Qingdao Marine Chemical Company (Qingdao, China).

The human NSCLC cell lines (NCI-H292, NCI-H441, NCI-H1781, NCI-H1975, and A549) were obtained from the American Type Culture Collection (ATCC). Antibodies against phospho-EGFR (Tyr1068) and EGFR were obtained from Abcom (Lake Placid, NY, USA). The anti-β-tubulin antibody was obtained from Proteintech (Rosemont, IL, USA). Anti-mouse IgG peroxidase-linked whole antibodies and anti-rabbit IgG peroxidase-linked species-specific

whole antibodies were obtained from Thermo Fisher Scientific (Waltham, MA, USA).

Synthesis of (2R,3R)-5,7-dihydroxy-2-(3′,4′,5′-trihydroxyphenyl) chroman-3-yl 3″,5″-dihydroxy-4″-(prop-2-yn-1-yloxy)benzoate **2**. To a solution of EGCG (458 mg, 1 mM) in dry DMF (5 mL), sodium hydride (60 mg, 1.5 mM) was added at 0 ºC under nitrogen. The mixture was stirred at room temperature for 0.5 h, propargyl bromide (0.1 mL, 1 mM) was quickly added, and the reaction mixture stirred at 80°C for 12 h. The solvent was evaporated under vacuum and the residue was purified by flash column chromatography on silica gel ($CHCl_3$/CH_3OH, 9:1) to afford the major product **2** (183 mg, 40%). ^{1}H-NMR (CD_3OD, 500 MHz): δ = 6.90 (s, 2H, $C^{2''}$–H, $C^{6''}$–H), 6.50 (s, 2H, $C^{2'}$–H, $C^{6'}$–H), 5.96 (s, 2H, C^6–H, C^8–H), 5.53 (m, 1H, C^3–H), 4.97 (d, 1H, J=4.6 Hz, C^2–H), 4.78 (d, 2H *J* = 2.4 Hz, C^{12}–CH_2), 3.29 (t, 1H, *J* = 1.6 Hz, C^{14}–H), 2.96 (dd, 1H, *J* = 4.6 Hz, 12.0 Hz, C^4–CH_a), 2.85 (dd, 1H, *J* = 4.6 Hz, 12.0 Hz, C^4–CH_b); ^{13}C-NMR (CD_3OD, 125 MHz): δ = 167.0 (C-11), 157.9 (C-7), 157.8 (C-5), 157.2 (C-9), 151.9 (C-3″, C-5″), 146.7 (C-3′, C-5′), 138.4 (C-4″), 133.8 (C-4′), 130.7 (C-1′), 127.1 (C-1″), 110.1 (C-2″, C-6″), 106.8 (C-2′, C-6′), 99.3 (C-10), 96.5 (C-6), 95.9 (C-8), 80.4 (C-2), 79.5 (*C*≡CH), 78.5 (C≡*C*H), 70.3 (C-3), 60.0 (C-14), 26.8 (C-4); ESIMS: *m/z* = 495 $[M-H]^-$.

Synthesis of (2R,3R)-5,7-dihydroxy-2-(3′,4′,5′-trihydroxyphenyl) chroman-3-yl 3″,5″-dihydroxy-4″-((1‴-β-((2S,3R,4S,5S,6R)-3‴, 4‴,5‴-trihydroxy-6‴-(hydroxymethyl)tetrahydro-2H-pyran-2-yl)-1H-1,2,3-triazol-4-yl)methoxy)benzoate **6**. To a solution of the glycosylated azide **5** (20.5 mg, 0.1 mM) and EGCG terminal alkyne **2** (49.6 mg, 0.1 mM) in THF (1.0 mL) and *t*-BuOH-H_2O (1:1, 1 mL) were added copper(II) acetate (1.8 mg, 0.01 mM) and sodium ascorbate (1.0 M in H_2O, 0.1 mL). The reaction mixture was stirred at room temperature for 2 h until the disappearance of the starting material was complete as indicated by TLC. The mixture was then evaporated and the residue was purified by column chromatography to afford the product compound **6** (52 mg, 75%). ^{1}H-NMR (CD_3OD, 500 MHz): δ = 8.11 (s, 1H, C^{14}–H), 6.91 (s, 2H, $C^{2''}$–H, $C^{6''}$–H), 6.53 (s, 2H, C2′-H, C6′-H), 5.96 (s, 1H, C^6–H), 5.95 (s, 1H, C^8–H), 5.58 (d, 1H, *J* = 7.6 Hz, $C^{1'''}$–H), 5.21 (m, 1H, C^3–H), 5.11 (s, 1H, C^2–H), 4.78 (d, 2H, *J* = 10.4 Hz, C^{12}–CH_2), 3.89–3.86 (m, 2H, C3‴-H, C2‴-H), 3.72–3.69 (m, 1H, C4‴-H–H), 3.57–3.53 (m, 2H, C6‴-H–CH_2), 3.51–3.48 (m, 1H, C5‴–H–H), 2.99–2.87 (m, 1H, C^4–CH_a), 2.85–2.82 (m, 1H, C^4–CH_b); ^{13}C-NMR (CD_3OD, 125 MHz): δ = 167.1 (C–11), 157.9 (C–7), 157.8 (C–5), 157.0 (C–9), 151.9 (C–3″, C–5″), 151.7 (C–3′, C–5′), 146.7 (C–13), 139.4(C–4″), 136.3 (C–4′), 127.0 (C–1′), 124.9 (C–1″), 124.9 (C–14), 110.4 (C–2″, C–6″), 106.9 (C–2′, C–6′), 106.8 (C–1‴),99.3 (C–10), 96.6 (C–6), 95.9 (C–8), 81.1 (C–2), 78.4 (C–3‴), 74.1 (C–4‴), 70.8 (C–2‴), 70.2 (C–12), 66.0 (C–5‴), 62.4 (C–3), 60.0 (C–6‴), 26.7 (C–4); ESIMS: *m/z* = 702 $[M+H]^+$.

Cell cultures

All the cell lines used in this study were cultured in RPMI-1640 medium (Thermo Fisher Scientific, Pittsburgh, PA, USA) supplemented with 50 IU/mL of penicillin, 1% of streptomycin (Solarbio, Beijing, China), and 10% of fetal bovine serum (HyClone, Los Angeles, CA, USA) at 37°C in a humidified 5% CO_2 incubator.

Cell viability assay

Cell viability was evaluated by the MTT assay. The NSCLC cells (NCI-H292, NCI-H441, NCI-H1781, A549, and NCI-H1975) were seeded in 96-well plates (5×10^4 cells/well) and then treated with compound **6** (0, 30, 60, 90, 120, or 150 μM), cisplatin/paclitaxel (12 μM), compound **6** (60 μM) plus varying cisplatin/paclitaxel (12 μM) for 48 h. Then, 20 μL of MTT was added to the each well and the plates were incubated for a further 4 h. After removal of the culture medium, the produced MTT formazan crystals were dissolved in 150 μL of dimethyl sulfoxide (DMSO). The optical density (OD) at 492 nm was measured using a microplate reader. The percentage of inhibition was calculated as follows: inhibition ratio (IR, %) = (1 − OD(sample)/OD(control)) × 100%.

WB analysis

Samples containing equal amounts of proteins as indicated in the text were resolved by sodium dodecyl sulfate (SDS) polyacrylamide gel electrophoresis and transferred to polyvinylidene fluoride (PVDF) membranes. The membranes were blocked with 5% bovine serum albumin (BSA) at room temperature for 1 h and then probed with primary antibodies overnight at 4°C and incubated with the horseradish peroxidase (HRP)-conjugated secondary antibodies for 1 h at room temperature. HRP was detected using a Pro-light HRP Chemiluminescent Kit (Tiangen Biotech, Beijing, China) and FluorChem E System (ProteinSimple, Santa Clara, CA, United States).

Stability assay

The H_2O_2 concentrations of EGCG and compound **6** were detected using a H_2O_2 Quantitative Assay Kit (Water-Compatible; Sangon Biotech Co., Ltd.). Briefly, 20 μL samples and 100 μL of working solution were added to each well of a 96-well microtiter plate (Thermo Scientific, Waltham, MA, USA) and incubated for 20 min on a rotary shaker at room temperature. The absorbance was recorded on a microplate reader at a wavelength of 595 nm.

Statistical analysis

All results are expressed as the mean ± the standard error of the mean (SEM) from three or more independent replicates. The data were statistically analyzed with either the Student's *t*-test or one-way analysis of variance (ANOVA). $p < 0.05$ was considered statistically significant. All of the statistical analyses were performed using GraphPad Prism 5.0 software (San Diego, CA, USA).

Declaration of conflicting interests

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References

1. Siegel RL, Miller KD and Ahmedin J. *CA Cancer J Clin* 2017; 67: 7.
2. Pignon JP, Tribodet H, Scagliotti GV, et al. *J Clin Oncol* 2008; 26: 3552.
3. Wangari-Talbot J and Hopper-Borge E. *J Can Res Updates* 2013; 2: 265.
4. Watanabe S, Minegishi Y, Yoshizawa H, et al. *J Thorac Oncol* 2014; 9: 189.
5. Garassino MC, Martelli O, Broggini M, et al. *Lancet Oncol* 2013; 14: 981.
6. Itchins M, Clarke S and Pavlakis N. *Transl Lung Cancer Res* 2018; 7: S39.
7. Jamieson ER and Lippard SJ. *Chem Rev* 1999; 99: 2467.
8. Szejniuk WM, Robles AI, McCulloch T, et al. *Pharmacogenomics J* 2019; 19: 5.
9. Garces ÁH, Dias MS, Paulino E, et al. *Cancer Chemoth Pharm* 2015; 75: 221.
10. Yang CS and Wang ZY. *J Nat Cancer Inst* 1993; 85: 1038.
11. Suzuki Y, Tsubono Y, Nakaya N, et al. *J Epidemiol* 2005; 15: 118.
12. Jin L, Li C, Xu Y, et al. *Oncol Rep* 2013; 29: 1983.
13. Chen H, Landen CN, Li Y, et al. *J Oncol* 2013; 2013: 1.
14. Surh YJ. *Nat Rev Cancer* 2003; 3: 768.
15. Wei R, Mao L, Xu P, et al. *Food Funct* 2018; 9: 5682.
16. Ma YC, Li C, Gao F, et al. *Oncol Rep* 2013; 31: 1343.
17. Zhang X, Wang J, Hu JM, et al. *Molecules* 2016; 21: 620.
18. Kitao S, Matsudo T, Sasaki M, et al. *Biosci Biotechnol Bioch* 1995; 59: 2167.
19. Wang J, Wang YN, Sun PY, et al. *BJSTR* 2019; 18: 13474.
20. Thirumurugan P, Matosiuk D and Jozwiak K. *Chem Rev* 2013; 113: 4905.
21. Sharma MG, Rajani DP and Patel HM. Roy Soc Open Sci 2017; 6: 170006.
22. Sharma MG, Vala RM, Patel DM, et al. ChemistrySelect 2018; 43: 12163.
23. Patel DM, Sharma MG, Vala RM, et al. *Bioorg Chem* 2019; 86: 137.
24. Patel DM, Vala RM, Sharma MG, et al. *ChemistrySelect* 2019; 3: 1031.
25. Sharma MG, Pandya J, Patel DM, et al. *Polycycl Aromat Comp* 2019; 5: 395.
26. Tamura M and Okai H. *Carbohyd Res* 1984; 133: 207.
27. Kolb HC, Finn MG and Sharpless KB. *Angew Chem Int Edit* 2001; 40: 2004.
28. Sequist LV, Soria JC, Goldman JW, et al. *N Engl J Med* 2015; 372: 1700.
29. Cross DA, Ashton SE, Ghiorghiu S, et al. *Cancer Discov* 2014; 4: 1046.
30. Liu TC, Jin X and Wang Y. *Am J Cancer Res* 2017; 7: 187.